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EVA copolymer matrix for intra-oral delivery of antimicrobial and antiviral agents

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Abstract Biocompatible ethylene vinyl acetate copolymer (EVA) was utilized to study the release of an antiviral drug (acyclovir (ACY)) and an antimicrobial drug (doxycycline hyclate (DOH)). Release of both drugs from EVA was measured individually and in combination. The effect of drug combination of DOH and ACY is presented. Additionally, the release rate of DOH after coating of the matrix with a different copolymer, in drug-loading with increasing loads of DOH, and with increases in temperature are also presented. The drugs incorporated in EVA films were prepared from the dry sheet obtained by solvent evaporation of polymer casting solutions with drugs. Drug release from the films was examined for about 12 days in distilled water at 37 °C. Changes in optical density were followed spectrophotometrically. The combination of ACY and DOH resulted in an increased release of ACY by about three times ($P < 0.001$) while DOH showed a decrease in rate of about two times compared to the individual release rates ($P = 0.008$). Increases in drug levels of DOH resulted in increases in drug release rates ($P = 0.001$). The release rate of DOH increased with temperature ($P = .001$; 27, 32, 37 and 42 °C were studied) and the energy of activation ($\Delta E^{\neq} = 56.69$ kJ/mol) was calculated using the Arrhenius equation for the diffusion of DOH molecules. Thus, the release rates of drugs were influenced by many factors: drug combination, coating the device, drug-loading, and temperature variation. Therefore it is proposed that controlling these variables should make it possible to obtain therapeutic levels of drugs released from drug loaded polymer, which may be beneficial in treating oral infections.

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Introduction

Polymeric drug delivery systems for release of antimicrobial, antiviral and other agents for treating oral infections are an ongoing area of research leading to clinical applications. In dentistry, drug-loaded polymeric materials to control C*andida albicans* are being used to avoid repeated mouth washes [1, 2]. Fluoride ion release from some orthodontic adhesive resins [3] and methacrylate-based polymers have been described [4]. Patel et al. reported that a tetrahydrofurfuryl methacrylate/poly (ethyl methacrylate) (THFMA)/PEM) cold cure polymer system could be used as a delivery system for chlorhexidine diacetate (CDA) and other drugs for the treatment of chronic C*andida* infections in immune suppressed or palliative care patients [5–7]. It was also reported that polymer-based drug delivery systems consisting of chitosan, poly (lactide-co-glycolide), and PMMA polymers were used to deliver chlorhexidine digluconate for the treatment of oral infections [8]. It was recently reported that an implantable delivery system based on a copolymer of lactic and glycolic acids was used to deliver antimicrobial agents for the treatment of periodontal disease [9]. In another study, a copolymer of MMA and HEMA was used to deliver chlorhexidine diacetate

intra-orally to treat oral infections [10]. Very recently, dental composites based on HEMA and dimethacrylate were used to deliver chlorhexidine diacetate in order to prevent bacterial microleakage in an in vitro model in which HEMA content in the composite was considered as responsible for controlling the release of chlorhexidine diacetate [11].

Prolonged delivery of antiviral and antimicrobial drugs within safe and effective exposures in the oral cavity is the principal goal in controlled release formulations [12, 13]. Tetracyclines have several therapeutic applications in dental practice because of their broad antibacterial spectrum, their efficient diffusion in bone and their inhibitory effect on collagenases and bone resorption [14, 15]. For these reasons, they are used widely in the treatment of various clinical types of periodontitis [16, 17]. However, prolonged oral administration leads to side effects such as digestive disturbances, enamel dysplasia, and tooth discoloring. In order to minimize these effects, research efforts have been devoted to the local and slow release of these antibiotics from polymeric materials used in the oral environment [18, 19]. Oral opportunistic infections are often persistent in immunocompromised patients and are intractable causing significant morbidity. Acyclovir has strong antiviral activity against herpes viruses, but it has poor availability when administered orally [20]. Secondary manifestations such as oral lesions, blisters, and fungal diseases can be treated intra-orally by the use of antiviral agents such as acyclovir from the intra-oral delivery system based on EVA. Therefore, a sustained release dosage form of acyclovir for buccal application was developed recently [21].

A simple method involving drug loaded biocompatible copolymer (ethylene vinyl acetate (EVA)) to release drugs in vitro at concentrations that have antiviral and antibacterial activity and at a relatively constant rate for an extended period of time is being studied in our laboratory [22, 23]. Development of an intraoral drug delivery system for the release of drugs should be useful to treat oral infections. The present study was designed to investigate the release of two drugs, the antiviral acyclovir (ACY) and an antibacterial, doxycycline hyclate (DOH), from drug-loaded EVA both individually and in combination. This work also examined the effect of coating, loading different levels of drugs, and temperature variation on the release rate of DOH. The use of combinations of these drugs may prove more effective in treating microbial and viral oral infections.

Materials and methods

EVA, (Elvax; Grade containing 40 & 32% vinyl acetate) (Du Pont, Wilmington, DE), Doxycycline hyclate powder (DOH), and Acyclovir (ACY) were from Sigma Chemical Company, St. Louis, MO and dichloromethane (Mallinckrodt Baker Inc. Spctr AR, Paris, KY).

Preparation of polymer thin films

Polymer casting solutions were prepared by dissolving EVA (VA 40%) copolymer beads and the drug in the ratio (40:1) in dichloromethane in a stoppered conical flask. Compositions of DOH/ACY ranging from 100/0, 75/25, 50/50, 25/75, and 0/100 (wt%) were used in the present study. Each solution was stirred with use of a magnetic stir rod at room temperature overnight and was then poured into a glass PYREX Petri-dish. For films containing DOH, it was necessary to place a non-stick material (the backing sheet of adhesive mailing labels) to prevent sticking in the bottom of the Petri dish; these backing papers contain no releasing agent, and no material was transferred to the films. The solution was then allowed to dry overnight in a fume hood to remove any solvent by evaporation at room temperature. Three samples of drug-loaded polymer thin square films of dimension 3 cm × 3 cm × 0.08 cm were cut from the dry films and used to follow the kinetics of drug release. The square films were suspended with two faces exposed in a volume of 10 mL distilled water at 37 °C to collect the drug released daily.

In order to study the effect of coating on the release rate of DOH, the dried thin DOH films held by forceps on one corner were dipped in a solution dichloromethane with EVA (VA 32%) and dried overnight. The thickness of the film before and after coating of the dried films was measured at different places by means of digital calipers. The effect of coating on the release of DOH at 37 °C was examined. We also measured the effect of temperature on the release rate of DOH at 27, 32, 37, and 42 °C. In addition, the effect of drug loading was studied with 1.0, 1.5, 2.0, 2.5, 5.0, 7.5 and 10.0 wt% DOH.

Fresh samples of 10 mL of the media were used daily for a period of 12 days and the concentrations of the drug released were determined by measuring the optical density spectrophotometrically (Hitachi U3010) at the respective wavelengths of maximum absorption (λ_{max} 344 nm for DOH and 253 nm for ACY). The aqueous release medium was exchanged every day and its drug concentration was determined spectrophotometrically. Drug release rates were determined (Tables 1–4) by taking the mean and standard deviation of rate measurements.

Scanning electron microscopy

DOH loaded EVA films with 2.5, 5, 7.5 and 10% were examined by scanning electron microscopy. Duplicate sets of samples were prepared. One set was prepared directly as received from the film production process. The second set

underwent an immersion procedure for 12 days in water. Sample films were cut into 2–3 mm squares and mounted onto aluminum specimen planchets. The samples were then coated with a thin film of gold/palladium (Polaron 5100). Samples were imaged with a JEOL JEM 6300 scanning electron microscope with 15 kV accelerating voltage. Digital images were made at 190×. Sets of 5 images were made for each drug loading condition (2.5–10%) with and without water immersion processing to be used for estimating the amount and size of surface porosity.

Statistical analysis

For each study, one-way analysis of variance (ANOVA) was applied to the drug release rates transformed to the log scale to achieve approximate normality and variance homogeneity. If the overall F-test for comparing drug load composition (or temperatures) was statistically significant at the 0.05 level, all post-hoc pair wise comparisons were tested with Bonferroni adjustment of *P*-values [24] with a statistically significant difference in rate pairs defined as *P*-value < 0.05/6 or 0.008.

Results

Table 1 shows the release rate of DOH and ACY at 37 °C in water as individual components and in combination. Figure 1a and b represents the cumulative release profiles with respect to time from day three onwards in order to omit the initial burst of drug release occurring within the first two days (Fig. 2a and b showing Initial Burst Release also). In the combinations as well as individual components (i.e., 100 wt% of each drug), the release rate of DOH is higher than that of ACY. The intermediate compositions of ACY/DOH (75/25, 50/50, 25/75) exhibited an increasing trend of ACY release whereas DOH exhibited a decreasing trend relative to the individual drugs alone. However, while each ACY combination had a statistically significantly higher release rate than the individual component (all $P < 0.001$), differences in the release rate of ACY among the combinations did not differ significantly. In the case of DOH, the release rate was less in the combination compared to 100 wt% DOH, and its release was in accordance with its proportion in the combination (overall ANOVA *P*-value = 0.008); statistically significant pair wise differences were found for 100 vs. 50 wt% ($P = 0.006$), and 100

Table 1 Composition of the drug load and release rate of the drugs ACY and DOH used in the EVA (VA 40%) matrix

Composition % of the drug load (2.5 wt% total)		Rate* of drug release at 37 °C μg/cm^2 · day	
ACY	DOH	ACY	DOH
100	0	0.769 (0.116)	–
75	25	2.062 (0.06)	3.817 (0.28)
50	50	2.139 (0.12)	4.2 (0.39)
25	75	2.299 (0.14)	6.12 (.61)
0	100	–	7.28 (.91)

*Rate expressed in terms of mean (SD)

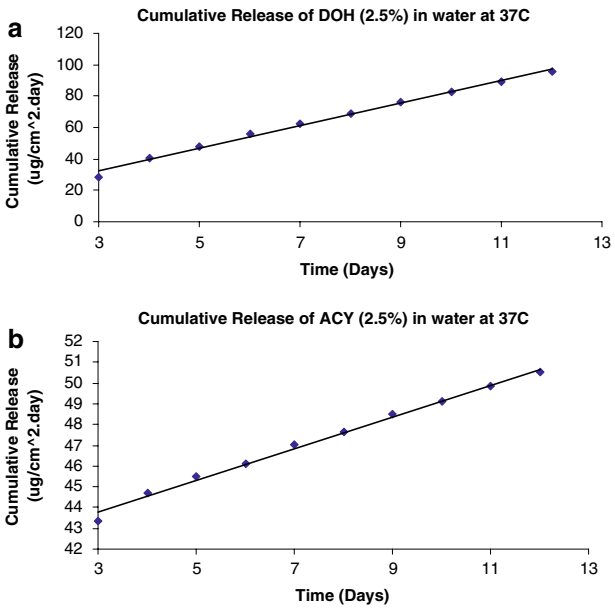

Fig. 1 (**a**) Time release (cumulative) profiles of DOH at 37 °C in water (**b**) Time release (cumulative) profiles of ACY at 37 °C in water

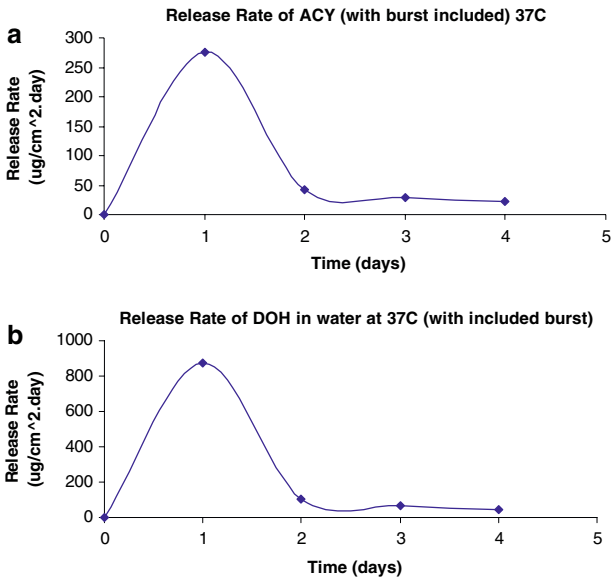

Fig. 2 (**a**) Time release profiles of ACY (Included Initial Burst) at 37 °C in water. (**b**) Time release profiles of DOH (Included Initial Burst) at 37 °C in water

vs. 25 wt% ($P = 0.003$), whereas differences between 25 vs. 75 wt% were nearly significant ($P = 0.009$). Figure 3a–c shows the relationship between the composition of ACY and DOH and their corresponding rates of release at 37 °C in water. All release studies exhibited an initial high release of drug followed by a much lower sustained release.

The release rate of DOH from the coated device is 1.51 μg/cm^2 · day, much less than the release from uncoated device (Table 2). The data in Table 3 summarizes the effect of loading DOH ranging from 1.0 to 10 wt% in EVA on the release rate of DOH. The data shows that the release rate increases as the drug load is increased (overall ANOVA $P = 0.001$); statistically significant differences (i.e., $P < 0.002$) were found for all pair wise comparisons except 1.0 vs. 1.5 wt%, 1.5 vs. 2.0 wt%, 2.5 vs. 5.0 wt%, and 7.5 vs. 10.0 wt%. Notwithstanding the failure to detect,

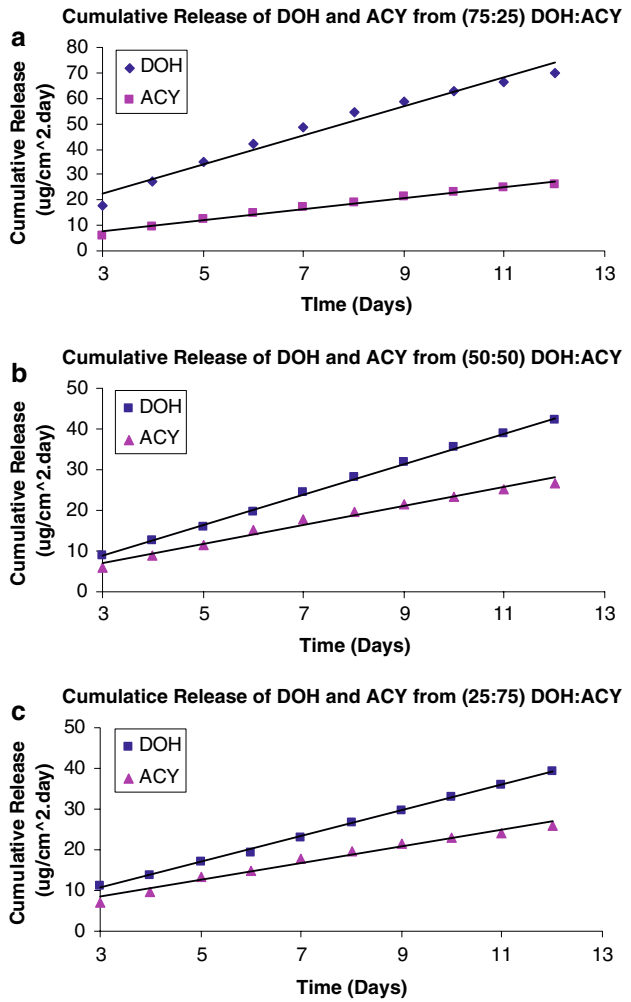

Fig. 3 (**a**) Time release (Cumulative Release) Profiles for the Samples Containing Intermediate Mixture of 75/25 DOH/ACY (**b**) Time Release (Cumulative Release) Profiles for the Samples Containing Intermediate Mixture of 50/50 DOH/ACY (**c**) Time Release (Cumulative Release) Profiles for the Samples Containing Intermediate Mixture of 25/75 DOH/ACY

Table 2 Release rate of DOH from uncoated and coated EVA films

Rate of drug release from uncoated film (μg/cm^2 · day) 10^{-12}(g/cm s))	Rate[a] of drug release from coated film (R) (μg/cm^2 · day)	Thickness of the coating (L) (cm)	Permeability $P = RL$
7.3 (.91)	1.51 (.29)	0.024 (.02)	0.41

[a] Rate expressed in terms of mean (SD)

Table 3 Release rate with increase in drug load in water

DOH drug load (wt%)	Release rate (μg/cm^2 · day)[a]
1.0	2.92 (.52)
1.5	3.44 (.28)
2.0	4.25 (.20)
2.5	7.28 (.91)
5.0	8.47 (.11)
7.5	13.7 (1.44)
10.0	18.42 (.93)

[a] Rate expressed in terms of mean (SD)

as statistically significant, differences in release rates for drug loads of comparable magnitude, a clear dose-response relationship exists; regressing release rate on DOH drug load shows that with every 1.0 wt% increase in DOH drug load, there is an estimated 1.67 (standard error, 0.09) μg/cm^2 · day increase in release rate ($R^2 = 0.96$ with quadratic DOH drug load effect not significant, $P = 0.48$).

The release rates of DOH at four different temperatures: 27, 32, 37, and 42 °C are shown in Table 4; the release rate of DOH increased with increase in temperature (ANOVA $P < 0.001$); statistically significant pair wise differences ($P < 0.006$) were found for all pairs of temperatures except 37 vs. 42 °C ($P = 0.22$). Energy of activation ($\Delta E^{\neq}$) for the diffusion process of DOH at these temperatures was calculated using the following Arrhenius equation (1). The energy of activation is a characteristic of the process of drug release and determines the influence of temperature on the rate of drug release (k) using the logarithmic form of Arrhenius Eq. (1) for the rate expression:

Table 4 DOH values of near constant release rates in water at 27, 32, 37, and 42 °C

Temperature (°C)	Release rate (μg/cm^2 · day)[a]
27	2.68 (.32)
32	4.57 (.011)
37	7.30 (.91)
42	8.23 (.001)

[a] Rate expressed in terms of mean (SD) $\Delta E^{\neq} = 56.69$ (kJ/mol) (From Arrhenius Equation)

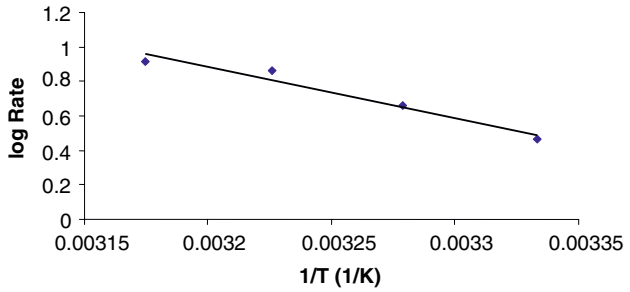

Fig. 4 Arrhenius plot between log rate vs. 1/T with reference to DOH in water

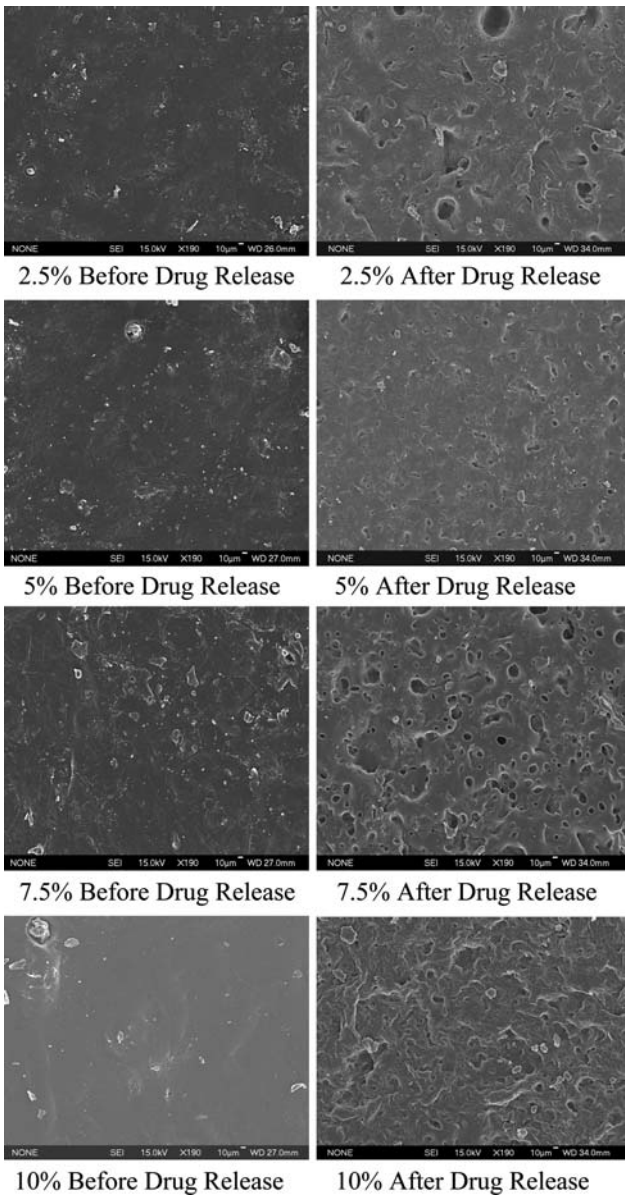

Fig. 5 Images of sample morphologies before and after drug release for different drug loadings of DOH (2.5–10%)

$$\log k = \log A - \frac{\Delta E^{\neq}}{(2.303 \times RT)} \quad (1)$$

Where k and A are respectively the rate of drug release and the frequency factor for the diffusion process, E is the energy of activation (cal mole^{-1}), T the absolute temperature, and R the gas constant (8.314 joules/mol).

The energy of activation is determined from the slope of the plot of log (k) vs. 1/T. From the slope of the straight line the equation (–ΔE/2.303R) = (–ΔE/4.57) is obtained. The energy of activation ($\Delta E^{\neq}$) for the diffusion of the drug molecule through the matrix was calculated to be 56.69 kJ/mol (Table 4 and Fig. 4).

Scanning electron microscopy

In these images the differences in image topography are evident. The immersed films have sharply delineated edges with large, well defined pores some of which appear to penetrate deeply (10s of micrometers). The immersed films are also about half again as thick as the unimmersed samples. The unimmersed films seemed fairly smooth with little details on the surface. Drug particles were distributed on the surface of the unimmersed samples. Immersed samples appeared very clean with fewer free surface particles visible, although partially embedded particles were often visible (Fig. 5).

Discussion

Discussion of our findings on rates of drug release is divided below among the following topics: Effect of Combination of Drugs, Effect of Coating, Effect of Loading, Effect of Temperature, and Scanning Electron Microscopy.

Effect of combination of drugs on release rate

Analysis of the data as seen in Table 1 and Fig. 3a–c revealed that ACY in the intermediate compositions (75/25, 50/50, 25/75) exhibited higher release rates than the ACY alone, while lower rates were observed for DOH release in these same intermediate compositions than the DOH alone. This could be attributed to drug-drug interactions and drug-polymer interactions (i.e. drug solubility in the matrix) which are among the various factors that play a critical role in the release of drugs. DOH exhibited consistently in combination higher release rates than those of ACY. This may be explained as due to lower solubility of the former (DOH) in the matrix than the latter (ACY).

It is of interest to note that the release rate of ACY increases with lower drug loadings (Table 1). The observed changes in drug release rates with reference to ACY may be explained as due to the molecular association phenomenon. The relatively lower drug release rate at higher drug loading is perhaps due to molecular association, whereas in the case of lower drug loading, the observed higher release rate may be due to the absence of such molecular association.

Effect of coating on the drug release rate

The release rate of a drug from an intra-oral drug delivery system can be influenced by coating the films with a different polymer [23, 25]. The reduction in the rate of drug release is attributed to an increase in the diffusion path length for the translocation of the drug molecules though the channels present in the matrix. In this study, the films were coated with an EVA copolymer of lower vinyl acetate content (i.e., 32%) and the data from Table 2 shows a decrease in the release rate of DOH from the coated device. This is consistent with our previous finding with reference to chlorhexidine diacetate that coating significantly reduces the rate of drug release [25]. It was observed that the rate of release of DOH from the uncoated system was drastically reduced by about 5 fold upon coating the system (uncoated 7.28 $\mu g/cm^2 \cdot$ day, coated 1.51 $\mu g/cm^2 \cdot$ day) as demonstrated in Table 2. Thus, when the film is coated with a copolymer, the coating will produce a drastic reduction in the rate of drug release. This is perhaps due to a lower solubility of drug in the copolymer with higher ethylene content (32% VA) and increase in the length of the diffusional path. Permeability was calculated for the coating of the film of thickness $L = 0.024$ cm assuming that this uncoated film exhibits a constant release rate $R = 1.51$ $\mu g/cm^2 \cdot$ day

Effect of loading on the rate of release

The release rate of DOH increased with increasing drug loading (wt%) in the polymer matrix as shown in Table 3 which shows a linear relationship between drug loading in the range from 1.0 to 10 wt% in EVA. It was reported earlier that the rate of drug release was affected by increases in the drug-loading of the polymer system [26]. Water diffuses into the matrix through the dispersed phase to dissolve the drug upon contact. The drug particles once dissolved leave behind pores in the polymer matrix. The drug molecules can then diffuse out through the interconnecting pores [25–27].

The experiments involving lower drug loadings (2.0%, 1.5%, 1.0%) for pure DOH resulted in decrease in the release rate, establishing the dominant effect of drug loading on the release rate in this system (Table 3).

Effect of temperature on the rate of release

It is generally known that an increase of temperature results in an increase in the rate of diffusion of molecules, either in liquids or solids [28, 29]. This can be extended to include the drug delivery process involving the diffusion of the drug molecules through the polymer matrix when immersed in the extracting medium. The data in Table 4 shows an increase in release rate of DOH with increase in temperature.

From the slope of the Arrhenius plot, the activation energy ($\Delta E^{\neq}$) was determined to be 56.69 kJ/mol. This represents the energy required for the translocation (diffusion) of DOH molecules through the channels present in the matrix. This is consistent with our previous observation made with reference to ACY for which the activation energy was found to be 68.82 kJ/mol [30].

The differences in energetics associated with ACY (68.82 kJ/mol) and DOH (56.69 kJ/mol) may be explained in terms of their relative interactions with the surrounding EVA matrix, since they do not appear to correlate with molecular weight. Thus, the higher $\Delta E^{\neq}$ value for ACY may be interpreted as due to stronger interactions between ACY molecules and the matrix than for DOH molecules.

It is well known that the activation energy associated with the diffusion process of a molecule through a liquid medium is generally not greater than 20.92 kJ/mol [28 p. 50]. This may interpreted as due to the weak interactions between the diffusing molecules and the surrounding medium. In the present context, it was observed that DOH molecules required an energy of activation of 56.69 kJ/mol for the translocation (diffusion) through the channels present in EVA (solid film), and even higher for ACY. These activation energy levels are certainly higher than that required for diffusion through a liquid medium, suggesting strong and tight interactions between the diffusing molecules and the surrounding solid medium.

Scanning electron microscopy

The SEM images in Fig. 5 are best understood by considering the likely mechanism of solvent-evaporation technique EVA films. When first dissolved in dichloromethane, the EVA beads dissolve into extended branched polymer chains that are essentially a one dimensional solid configuration. With 24 h stirring, the drug (DOH) may be homogeneously dispersed, but as stirring is discontinued, and evaporation proceeds, the distribution of drug becomes significantly non-homogeneous. There is a significant heterogeneity that can be seen visually and felt in the final dried film, such that the bottom side that was in contact with the Petri dish has visible clusters of drug (perhaps crystals but at least conglomerates of drug-rich particles

near the bottom surface) and the surface feels rough to the touch in comparison to the top surface. By SEM, the actual bottom surface of the film prior to immersion in water appears relatively smooth, probably resembling the surface of the Petri dish. After immersion, it is reasonable to assume the drug-rich deposits begin to dissolve into the surrounding aqueous medium, initially producing pits, and then deeper cavities that can be loosely described as pores. In analogy to rock formations, there could also be "veins" or channels of drug connected with the surface that slowly dissolve leaving aqueous pathways to continue slowly allowing drug to escape from the film over extended periods of time. While it is thus difficult to describe the mechanism of drug release in classic terms like Fickian diffusion since it is far from an ideal, homogeneously disperse system, it is nonetheless remarkable that the drug release is essentially linear with time after the first two days of aqueous exposure.

Conclusions

Several ways of increasing or decreasing drug release from drug-loaded polymers were examined. Our results suggest the following generalizations: (1) The release of a drug from a drug-loaded polymer is influenced by drug-loading with the two drugs, the release of some drugs is increased while others may be decreased when compared to the drug release from a single drug loaded polymer; (2) The release of drugs can be reduced by coating a drug loaded polymer with another copolymer; (3) Increasing the amount of drug in the drug loaded polymer increases the release of the drug in the media; (4) Increasing the temperature of the extracting media increases the rate of release. These findings suggest ways to adjust the release rate of drugs from drug loaded polymers to levels appropriate for optimal antiviral and antimicrobial activity.

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